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Intrauterine Cortisol, Aldosterone, and Corticosteroid Binding Globulin-Like Activity during Early Porcine Pregnancy and the Estrous Cycle[1]

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ABSTRACT

Studies were conducted to determine whether the corticosteroids cortisol and aldosterone, and corticosteroid-binding globulin (CBG) were present in the porcine early-embryonic environment. Cortisol was measured in uterine flushings from white crossbred gilts at Days 7, 10, 13, and 16 of the estrous cycle and pregnancy. Total content of cortisol increased ($p < 0.01$) between Days 13 and 16, and immunoreactive CBG (ir-CBG) increased ($p < 0.01$) between Days 10 and 13, in both cyclic and pregnant gilts. In a separate study with Chinese Meishan gilts, total cortisol and aldosterone content of uterine flushings increased ($p < 0.02$) between Days 10 and 15 of the estrous cycle and pregnancy.

In another study with white crossbred gilts, CBG-like binding activity in uterine flushings was low at Day 10, then increased over 100-fold at Day 15 ($p < 0.01$). However, levels of CBG-like binding activity on Day 15 were 100-fold lower than those of ir-CBG measured in the previous study and could bind less than 4% of the uterine luminal cortisol. Differences between ir-CBG and CBG binding might be due to the ability of the CBG antibody to recognize either biologically inactive CBG or structurally similar molecules.

CBG-like binding activity, which appeared unrelated to glucocorticoid receptors, was also present in the endometrial cytosol of white crossbred gilts. Concentrations (fmol/mg protein) of endometrial CBG-like activity decreased ($p = 0.03$) between Days 10 and 15 of the estrous cycle and pregnancy, did not differ with reproductive status, and on Day 15 were comparable to concentrations in uterine flushings but threefold lower ($p < 0.01$) than those in the serum. Equilibrium dissociation constants for CBG-like binding activities were comparable among the three locations.

These studies indicate that corticosteroids are present—primarily in the free form—within the porcine uterine lumen and could influence early porcine conceptus development. Endometrial CBG-like binding activity could mediate actions of cortisol or progesterone on uterine function.

INTRODUCTION

In swine, as in other species, plasma glucocorticoids, such as cortisol, regulate final maturational changes in numerous tissues near term [1, 2]. Cortisol is present in fetal pig plasma by Day 50 of gestation [3] and is present within the whole embryo as early as Day 25 [4]. The developmental role of cortisol at these earlier ages in swine is not well understood but at Day 50 could include the control of differentiation of adipocytes [5, 6]. Glucocorticoids regulate gene expression [7] of elements such as the proto-oncogenes [8] and insulin-like growth factors [9, 10] that are associated with cellular proliferation and differentiation. Hence, glucocorticoids could influence prenatal development, not only near term but also throughout gestation via multiple mechanisms.

Corticosteroid-binding globulin (CBG), a monomeric glycoprotein, is a member of the serine protease inhibitor (SERPIN) superfamily of proteins [11]. Although it is predominantly produced in the liver and secreted into the blood, CBG mRNA has also been detected in extrahepatic tissues [12–14], including human endometrium [15]. It binds with high affinity to glucocorticoids and with approximately tenfold less affinity to progesterone [16]. Traditionally, it is thought that CBG-bound steroid is biologically inactive and that only free steroid is available to tissues to initiate a biological response [16, 17]. Hence, CBG-bound steroid may act as a reservoir of biologically inactive steroid that can dissociate and become readily available to target tissues [17]. More recent evidence suggests that CBG may provide glucocorticoids to specific sites by virtue of cellular CBG receptors or via enzymatic cleavage of CBG with associated release of steroid [17].

In order to affect early porcine conceptus development, glucocorticoids such as cortisol must be present within the embryos or in the uterine environment. Therefore, the present studies were conducted to determine whether cortisol is present within the uterine lumen during early pregnancy and to compare levels with those present during the estrous cycle. Because CBG can greatly affect the biological activity of cortisol, it also was measured within the uterine lumen and endometrium.

MATERIALS AND METHODS

Animals and Tissue Collections

In the first study, white crossbred gilts (10–12 mo of age) were maintained in groups of 10–20 in indoor swine facilities and were observed daily for behavioral estrus for two or three estrous cycles. Gilts were then randomly assigned to status group (cyclic or pregnant) and to sampling day (Day 7, 10, 13, or 16 of the estrous cycle; Day 7, 10, 13, 16, or 19 of pregnancy; n = 4–7 gilts for each status and day combination). In a second study, white crossbred gilts (9–10 mo of age) were maintained and observed for behavioral estrus as noted above and randomly assigned to status (cyclic or pregnant) and sampling day (Day 10 or 15; n = 4–7 for each status and day combination). In a third study, Chinese Meishan gilts (9–10 mo of age) were also maintained and observed for behavioral estrus as noted above. These gilts were randomly assigned to status (cyclic or pregnant) and sampling day (Day 10, 13, or 15; n = 4–6 for each status and day combination). For all studies, Day 0 was designated as the first day of behavioral estrus. Appropriate gilts were naturally bred to boars of the same

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breed. Blood samples were obtained at slaughter, the reproductive tract was immediately removed, and each uterine horn was flushed with 20 ml of Minimum Essential Medium. The volume of uterine flushing recovered from each horn was measured, and the presence or absence of conceptuses in each horn was confirmed. In the first study, uterine flushings from each horn were stored and analyzed separately. For ELISA measurements of CBG, data for one horn in each of four gilts were missing. Therefore, data from the remaining uterine horn were doubled to provide information on a per paired uterine horn basis. In the second and third studies, flushings from the two horns were combined. After centrifugation, uterine flushing supernatants and serum samples were frozen at −20°C until assayed. In the second and third studies, samples of endometrium were taken from random sites of the uterine horns, rapidly frozen in liquid nitrogen, and stored frozen at −80°C until used in assays. All procedures involving use and slaughter of animals were approved by our institutional Animal Care and Use Committee.

Cortisol, Progesterone, and Aldosterone RIA Procedures

Cortisol in uterine flushings was measured using antibody-coated tubes and ^{125}I-cortisol (Diagnostic Products Corp., Los Angeles, CA) after extraction of 8 ml of uterine flushing with ethyl acetate and using [1,2,6,7-^{3}H]cortisol (Amersham, Arlington Heights, IL) to measure procedural losses. Extracted cortisol and cortisol standards were resolubilized in buffer (0.01 M sodium phosphate, 0.15 M NaCl, 0.1% sodium azide, 0.1% gelatin, pH 7.4; PBSG). Accuracy of estimates after correction for procedural losses was 93.5%. A plot of expected versus measured cortisol had a slope (b = 0.99) that did not differ from 1 ($p > 0.5$). Serial dilutions of uterine flushing extract provided a slope (b = −0.77) that did not differ from that of the standard curve (b = −0.87; $p > 0.05$). The average within-assay coefficient of variability (CV) for duplicate estimates was 1.5%, and the average interassay CV was 2.7%. The sensitivity of this assay as measured by the lowest standard in the linear range of the standard curve was 0.153 ng/tube.

Progesterone in uterine flushings was directly measured using antibody-coated tubes and ^{125}I-progesterone (Diagnostic Systems Laboratories, Houston, TX). Average accuracy of this procedure was 101.6%, and a plot of expected versus measured levels had a slope (b = 0.92) that did not differ from 1 ($p > 0.05$). In nine of the gilts, progesterone in flushings from one or both uterine horns was below the sensitivity of the assay, measured in the largest possible volume for this assay procedure. Extraction of these uterine flushings and subsequent concentration to increase sensitivity could not be conducted because insufficient uterine flushing volume remained. To allow for statistical analysis, these latter samples were assigned the value of the lowest progesterone standard (250 pg/ml). Serum progesterone was measured using the same reagents, but after extraction with heptane and use of [1,2,6,7,21-^{3}H]progesterone to measure procedural losses. Accuracy of estimates for this procedure was 97.4%, and a slope of expected versus measured values (b = 0.92) did not differ from 1 ($p > 0.2$). Serum progesterone concentrations were measured in a single assay with an average within-assay CV of 4.6%. Progesterone in uterine flushings was also measured in a single assay with an intraassay CV of 1.8%. The sensitivity of this assay was 6.14 pg/tube.

Aldosterone in uterine flushings from Meishan gilts was measured using antibody-coated tubes and ^{125}I-aldosterone (Diagnostic Products Corporation) after extraction of 8 ml of uterine flushing with ethyl acetate and using [1,2-^{3}H]aldosterone (Amersham) to measure procedural losses. Extracted aldosterone and standards were resolubilized in PBSG. Accuracy of this procedure after correcting for procedural losses was 87.8%. A plot of expected versus measured aldosterone had a slope (b = 1.08) that did not differ from 1 ($p > 0.5$). Serial dilutions of uterine flushing extract provided a slope (b = −0.87) that did not differ from that of the standard curve (b = −0.89; $p > 0.05$). The average within-assay CV for duplicate estimates was 1.9%; the interassay CV was 11.6%. The sensitivity of this assay as measured by the lowest standard in the linear range of the standard curve was 7.5 pg/tube.

CBG Assay Procedures

Mass of CBG in uterine flushings was measured by an ELISA using a rabbit anti-porcine CBG antibody [18]. One milliliter of uterine flushing was exchanged into a sodium, potassium phosphate-buffered saline (pH 7.4) using Centricon-10 concentrators (Amicon, Beverly, MA). A sample was assayed on each plate (2–6 wells per plate) to assess variability. The average CV for absorbance of replicate wells within a plate was 1.99%. The CV of the mean absorbance between plates (total of 27 wells, 6 plates) was 2.1%. Sample absorbances were compared with those of wells containing purified porcine CBG at 0.16–160 ng/well. As the antibody to porcine CBG might recognize and bind to other members of the SERPIN superfamily of proteins known to be present in the uterus in high concentrations [19–21], binding specificity of the antibody to some of these proteins was characterized (Fig. 1). No cross-reactivity with other SERPINs (Sigma Chemical Co., St. Louis, MO), which included human α-$_1$-antitrypsin (0.1–10 μg), human α-$_1$-antichymostrypsin (0.1–10 μg), porcine antithrombin III (0.1–1.25 μg), human T_4 binding globulin (0.1–1 μg), and porcine angiotensinogen (0.1–1 μg), was apparent (Fig. 1A). There was, however, an average of 0.06% cross-reactivity with uteroferrin-associated protein (also a SERPIN) [22] purified according to Baumbach and coworkers [21] in amounts ranging from 0.225 to 11.25 μg (Fig. 1B).

Biological activity of uterine flushing-associated CBG was assessed by measurement of binding of [1,2,6,7-^{3}H]cortisol to uterine flushing exchanged into buffer (0.01 M sodium phosphate, 0.15 M NaCl, 0.1% sodium azide, 250 units aprotinin [ICN Biomedicals Inc., Aurora, OH] per ml of buffer, pH 7.4, PBS) using Centricon-10 or Centriprep-10 concentrators. Endogenous steroids were then removed by treatment with dextran-coated charcoal (250 mg charcoal and 25 mg dextran per 5–6 ml of uterine flushing) at 40°C for 30 min. Aliquots were taken for modified Lowry protein determinations [23]; then samples were adjusted to 0.1% gelatin using a 1% gelatin solution in PBS. For measurement of CBG in serum, endogenous steroids were removed from serum as noted above, then diluted 100-fold with PBSG before assay. For CBG measurements in endometrium, approximately 2 g of endometrial samples were minced, washed three times in 20 ml PBS without aprotinin to remove any blood, and then homogenized at a concentration of 1 g/5 ml in PBS with aprotinin. Homogenates were centrifuged at 110 000 × g_{av} for 1 h at 4°C. Endogenous steroids were removed from the 110 000 × g_{av} supernatant (soluble cytosol) as previously described, then di-

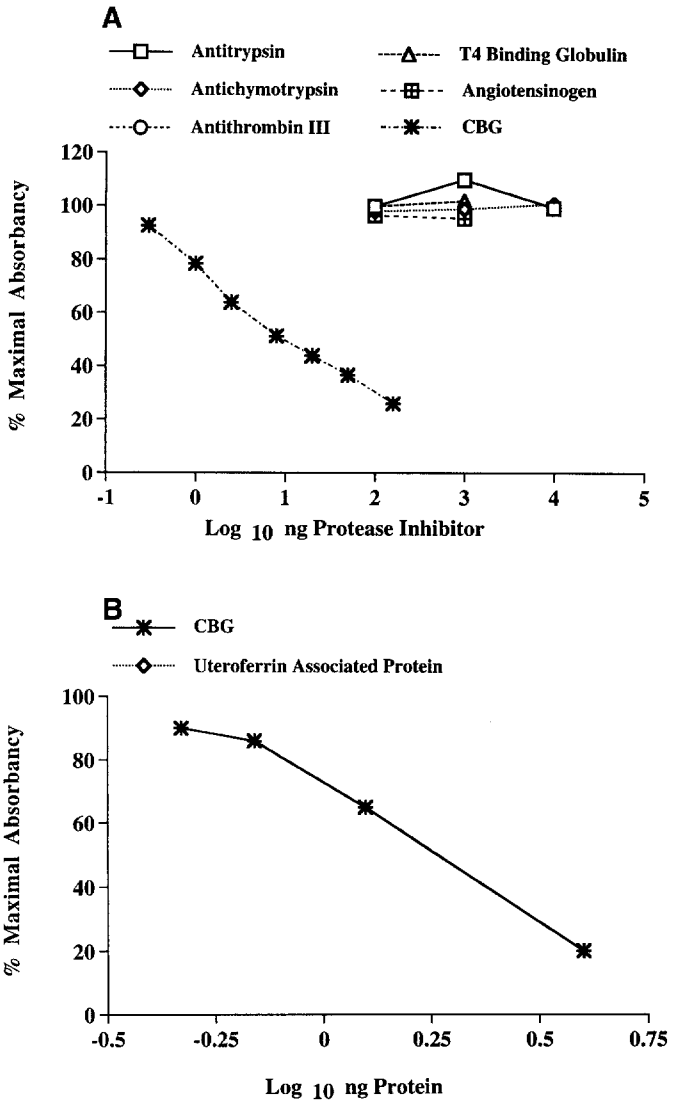

FIG. 1. A) Cross-reactivity of SERPINs with the antibody to porcine CBG. B) Cross-reactivity of the SERPIN uteroferrin-associated protein with the antibody to porcine CBG compared with CBG. All data represent the absorbancy of protein at each concentration of inhibitor divided by absorbancy in the absence of any competing protein multiplied by 100.

luted fivefold with PBSG. At this point, cytosol preparations were analyzed for the presence of hemoglobin via use of a blood gas analyzer (CIBA Corning Diagnostic Corp., Medfield, MA) as a measure of blood contamination. The sensitivity of this procedure relative to the amount of hemoglobin normally found in pig blood was such that as little as 0.13 μl of blood would be detectable. Uterine flushings and endometrial cytosol were incubated for 5 h at 4°C and serum for 2 h at 4°C with multiple concentrations of labeled and unlabeled cortisol (0.06–920 nM) to produce saturation curves. Bound and free cortisol were separated via use of dextran-coated charcoal as previously reported for CBG measurements in plasma of neonatal pigs [24]. Under the assay conditions used, binding achieved steady-state conditions at the time intervals used for each tissue and exhibited a linear increase in binding with increasing protein concentrations (data not shown). The interassay CV was 6.3%; the intraassay CV of duplicate samples for 8–10 different cortisol concentrations for each sample was 2.8%.

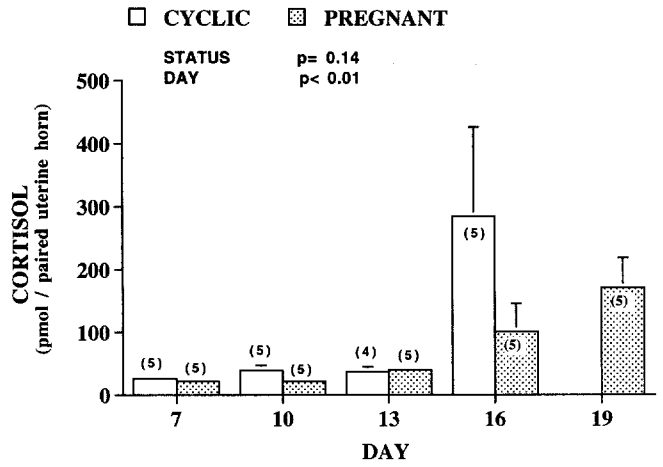

FIG. 2. Total content of cortisol in uterine flushings from white crossbred gilts during the estrous cycle and pregnancy. Each bar represents the mean + SEM of the number of gilts in parentheses. There was no status × day interaction ($p = 0.48$). Statistics were conducted on $\log_{10}$ transformed data. Some error bars are too small to be represented at the scale presented.

Statistical Analyses

The General Linear Models procedure of the Statistical Analysis System [25] was used for all analyses. For analysis of main effects and interactions, a two-way analysis of variance (ANOVA) was conducted. If interactions were significant, then a priori single degree of freedom comparisons were conducted [26]. All data were tested for homogeneity of variance with an F_{max} test, and data were transformed to a log or square-root function where necessary to fulfill assumptions of ANOVA. In all tables and graphs, the original untransformed data are presented. A probability level of ≤ 0.05 was considered significant. Saturation isotherms were analyzed by Scatchard analysis via use of the program LIGAND [27]. In these analyses, nonspecific binding was not subtracted from the data. Rather, the LIGAND program computed the nonspecific binding as a data-determined parameter based on binding present at high concentrations of unlabeled cortisol [27].

RESULTS

Uterine flushing volumes recovered per pair of uterine horns did not differ with status ($p = 0.14$). However, because of endogenous luminal fluids, recovered volumes on Days 16 and 19 exceeded ($p < 0.01$) those on earlier days. To obviate problems with this variable dilution of luminal solutes, most data are presented on a per paired uterine horn basis. However, whether uterine data are presented on a total content or concentration (pmol/ml; data not shown) basis, the interpretation is the same.

Cortisol content of uterine flushings (pmol/paired uterine horns) was low on Day 7 in both cyclic and pregnant white crossbred gilts (Fig. 2) and remained relatively constant through Day 13. Cortisol then increased ($p < 0.01$) 6.7-fold in cyclic gilts by Day 16 (Fig. 2) and fourfold ($p < 0.01$) in pregnant gilts by Day 19. Cortisol content of uterine flushings did not differ between pregnant and cyclic gilts ($p = 0.14$).

In order to assess the specificity of changes in intrauterine cortisol, progesterone was also measured. Progesterone content of uterine flushings was constant in cyclic white crossbred gilts on the days measured (Fig. 3A). In pregnant

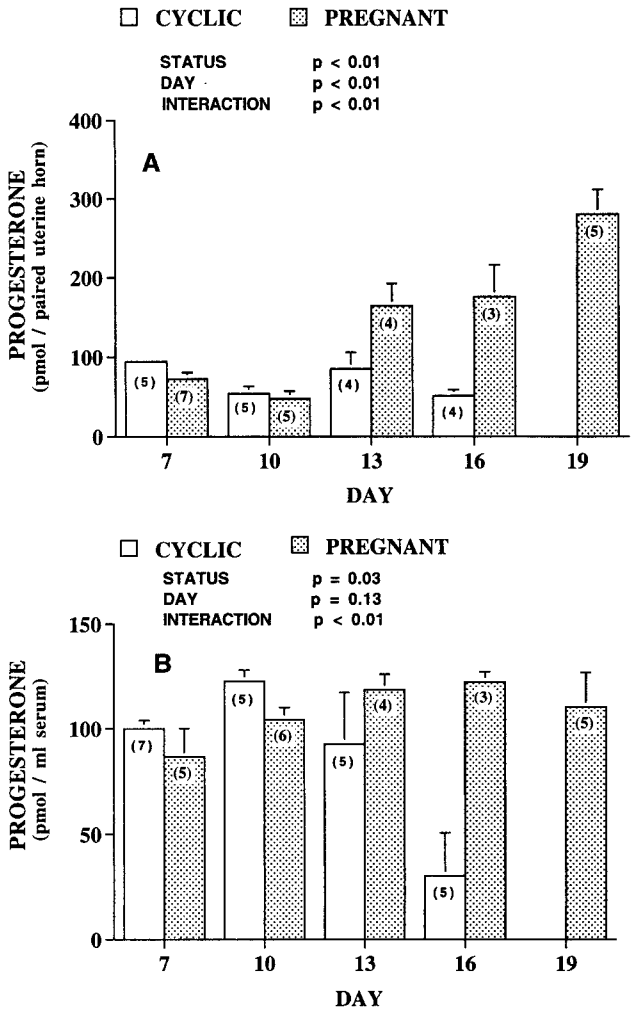

FIG. 3. **A**) Total content of progesterone in uterine flushings from white crossbred gilts during the estrous cycle and pregnancy. **B**) Serum progesterone concentrations in white crossbred gilts during the estrous cycle and pregnancy. Each bar represents the mean + SEM of the number of gilts in parentheses. Some error bars are too small to be represented at the scale presented.

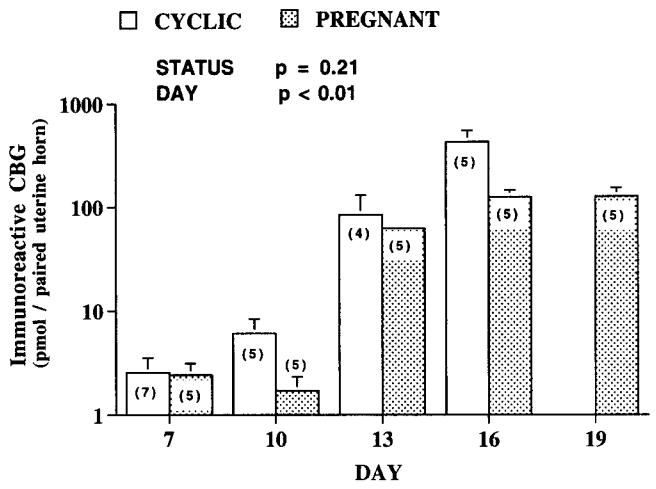

FIG. 4. Total immunoassayable CBG in uterine flushings from white crossbred gilts during the estrous cycle and pregnancy. Each bar represents the mean + SEM of the number of gilts in parentheses. There was no status × day interaction ($p = 0.10$). Statistics were conducted on $\log_{10}$ transformed data. Some error bars are too small to be represented at the scale presented.

gilts, uterine progesterone increased after Day 10 to reach the highest levels on Day 19 ($p < 0.01$). On Days 13 and 16, progesterone content of uterine flushings from pregnant gilts exceeded that of cyclic gilts ($p < 0.01$). Serum progesterone concentrations (Fig. 3B) were high and similar between Days 7 and 10. In cyclic gilts, serum progesterone concentrations decreased between Days 13 and 16. In pregnant gilts, progesterone concentrations did not differ among days ($p = 0.13$). Only on Day 16 did progesterone concentrations in pregnant gilts exceed those measured in cyclic gilts ($p < 0.01$). There were no correlations ($p = 0.32$) between serum and uterine flushing progesterone.

Immunoreactive CBG (ir-CBG) content of uterine flushings was low and relatively constant on Days 7 and 10 of the estrous cycle and pregnancy (Fig. 4). Between Days 10 and 13, however, ir-CBG increased 13-fold in cyclic gilts and 36-fold in pregnant gilts ($p < 0.01$). In cyclic gilts, ir-CBG increased further between Days 13 and 16, whereas in pregnant gilts, ir-CBG in uterine flushings did not vary significantly after Day 13 ($p > 0.05$). Only on Day 10 were the numerical differences between cyclic and pregnant gilts significant ($p < 0.05$).

The binding capacity of CBG in uterine flushings was determined in undiluted uterine flushings (Eagles MEM) after removal of endogenous steroids by charcoal treatment and using procedures previously validated for measurement of plasma CBG in neonatal pigs [24]. Scatchard analyses of binding assays from pregnant and cyclic gilts at Day 16 indicated low levels of binding activity (0.25 ± 0.05 and 2.2 ± 1.0 pmol per pair of uterine horns, respectively; n = 3 for each status). Such binding levels were 200- to 500-fold lower than those determined by ELISA (Fig. 4). Because of the discrepancy between ELISA and binding results, the binding assay was checked and revalidated, and procedures were optimized for all compartments measured (uterine flushings, endometrium, serum; *Materials and Methods* section). However, because of multiple measures conducted on these uterine flushings [28], the remaining flushings were insufficient to allow for further characterization of cortisol binding activity. Hence, in a second study using white crossbred gilts, samples were collected on Days 10 and 15, when binding of cortisol to CBG should be near its minimum and maximum levels.

As noted in the previous study, total content of cortisol in uterine flushings increased ($p = 0.03$) between Days 10 and 15 (Fig. 5A) and did not differ between cyclic and pregnant gilts ($p = 0.22$). This increased content of cortisol was most apparent in pregnant gilts. The CBG-like binding activity was below the detectable limit in 3 of the 4 pregnant and cyclic gilts at Day 10 (Fig. 5B). By Day 15, total CBG-like binding activity in uterine flushings increased 113-fold in cyclic and 166-fold in pregnant gilts (Fig. 5B). Such levels of CBG-like activity on Day 15 could bind with only 3.7% of intraluminal cortisol. However, if expressed as fmol/mg protein, the fold increases—while substantial—are not as large because of the concomitant increase in uterine flushing protein (Table 1). In contrast to that found in uterine flushings, endometrial cytosol binding of cortisol was highest on Day 10, then decreased ($p = 0.03$) by Day 15 (Table 1). Endometrial cytosol protein also decreased ($p < 0.01$) between Days 10 and 15 (Table 1). Hemoglobin was not measurable in any of the cytosol preparations (data not shown). Serum-associated CBG binding activity was constant ($p = 0.86$) between Days 10 and 15 (Table 1) and did not differ between pregnant and cyclic gilts ($p = 0.53$).

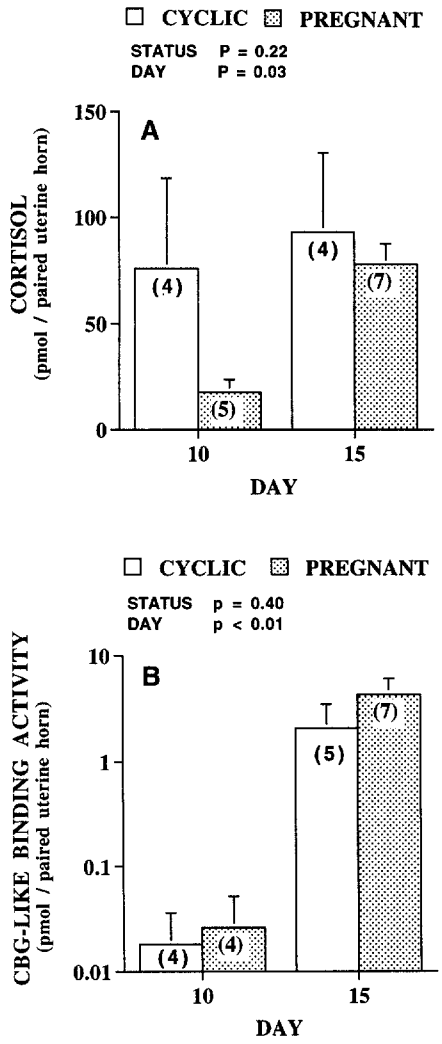

FIG. 5. **A**) Total cortisol content in uterine flushings from white crossbred gilts during the estrous cycle and pregnancy. There was no status × day interaction ($p = 0.10$). Statistics were conducted on $\log_{10}$ transformed data. **B**) Total CBG-like binding activity in uterine flushings from white crossbred gilts during the estrous cycle and pregnancy. Binding was measured in multiple concentrations of labeled and unlabeled cortisol, and maximal binding was determined by Scatchard analysis using the computer program LIGAND. For Day 10 cyclic and pregnant gilts, 3 of 4 gilts had no measurable activity and were assigned values of zero for statistical evaluations. There was no status × day interaction ($p = 0.41$). Statistics were conducted on $\log_{10}$ transformed data. Each bar represents the mean + SEM of the number of gilts in parentheses.

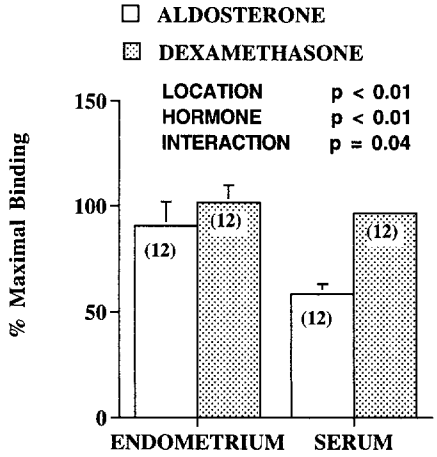

FIG. 6. Comparison of the ability of excess aldosterone (100 ng, 926 nM) and dexamethasone (100 ng, 850 nM) to compete with tritiated cortisol for CBG-like binding activity in serum and endometrial cytosol of cyclic and pregnant white crossbred gilts. Data are combined for Days 10 and 15 and for reproductive status. Data are expressed as percentage of maximal binding (total counts per minute [cpm] bound in the presence of competing steroid minus cpm bound in presence of excess cortisol [100 ng, 918 nM; i.e., nonspecific binding] divided by cpm bound in absence of competing steroid minus nonspecific binding). Each bar represents the mean + SEM of the number of gilts in parentheses.

There were also no differences in total serum protein associated with reproductive status ($p = 0.28$) or day ($p = 0.29$), and serum protein concentrations for all gilts averaged 9.73 g/100 ml. When compared on a per unit of protein basis on Day 10, irrespective of status ($p = 0.72$), there was no difference between CBG-like binding in serum and endometrium cytosol, but both were higher ($p < 0.01$) than binding in uterine flushings. On Day 15, CBG-like binding in uterine flushings and endometrial cytosol were similar, and both were less ($p < 0.01$) than that found in serum (Table 1). Equilibrium dissociation constants (K_d) of CBG-like binding with cortisol (Table 1) did not differ with day of gestation, reproductive status, or location of the binding (uterine flushing, endometrium, or serum). However, preliminary studies from two gilts indicated that K_d of CBG-like binding activity for progesterone in serum (2.39×10^{-7} M) and endometrial cytosol (1.39×10^{-7} M) were over 30-fold higher than K_d for cortisol measured concomitantly.

Because of the possibility that binding of cortisol in the endometrial cytosol represented binding to cellular glucocorticoid receptors, competition of excess dexamethasone and aldosterone (after subtracting out nonspecific binding) with labeled cortisol was measured and compared with that found in the serum (Fig. 6). In neither serum nor endometrial cytosol did dexamethasone compete with labeled cortisol. In the endometrial cytosol, binding of labeled cortisol in the presence of excess aldosterone did not differ from that in the presence of excess dexamethasone ($p = 0.22$). However in 1% serum, excess aldosterone effectively competed for approximately 42% of labeled cortisol, and binding of labeled cortisol in the presence of aldosterone was less than that in the presence of dexamethasone ($p < 0.01$).

To determine whether intrauterine changes in cortisol were breed-specific, its presence in uterine flushings from Chinese Meishan gilts was measured. Further, examination of another—potentially physiologically relevant—steroid of adrenal origin seemed prudent; therefore, aldosterone was also measured. Total uterine content of cortisol increased approximately 52% ($p = 0.03$) in both cyclic and pregnant Meishan gilts between Days 10 and 13 (Fig. 7A) and did not change thereafter. Aldosterone content of uterine flushings was highly variable, and therefore, increases in aldosterone did not attain statistical significance ($p = 0.02$) until Day 15 (Fig. 6B). Differences between cyclic and pregnant gilts were not apparent ($p = 0.99$). Aldosterone content was approximately 130-fold less than cortisol content.

DISCUSSION

To our knowledge, these data are the first for any species that demonstrate naturally occurring increases in uterine luminal corticosteroids (both cortisol and aldosterone) during the estrous cycle and early pregnancy. The highest intrauterine levels of cortisol measured in the current study are approximately one-third to one-half those measured on Day

TABLE 1. Uterine and serum measures in white crossbred gilts.

	Cyclic		Pregnant		Statistical evaluation		
Parameter	Day 10	Day 15	Day 10	Day 15	Status	Day	Interaction
Measure	n = 3–4	n = 3–5	n = 3–4	n = 7			
mg Protein/paired uterine horn	16.3 ± 5.4	123.4 ± 30.0	16.2 ± 4.8	87.5 ± 8.7	0.88	0.01	0.60[a]
Uterine flushing CBG-like binding (fmol/mg protein)	1.1 ± 1.0[b]	29.1 ± 21.9	1.4 ± 1.4[b]	65.4 ± 31.6	0.36	0.01	0.40[a]
Endometrium CBG-like binding (fmol/mg protein)	128.9 ± 55.2	51.8 ± 19.6	108.3 ± 22.8	82.2 ± 13.0	0.11	0.03	0.81[a]
Endometrial cytosol protein (mg/g tissue)	13.3 ± 1.6	7.9 ± 1.2	13.8 ± 0.7	11.2 ± 0.6	0.09	0.01	0.20
Serum CBG binding (pmol/ml)	50.5 ± 25.7	33.4 ± 7.9	28.2 ± 4.9	20.7 ± 2.8	0.38	0.67	0.61[a]
Serum CBG binding (fmol/mg protein)	517.0 ± 289.2	331.6 ± 46.5	297.3 ± 64.7	242.5 ± 41.9	0.53	0.86	0.68[a]
Equilibrium dissociation constants (K_d) (10^{-10} M)							
Uterine flushing	—	3.6 ± 1.5	—	5.1 ± 0.7	0.36	—	—
Endometrial cytosol	3.6 ± 0.7	2.3 ± 0.8	4.4 ± 1.4	5.2 ± 1.0	0.10	0.78	0.35
Serum	3.6 ± 0.7	3.2 ± 0.7	4.2 ± 1.7	7.6 ± 2.1	0.39	0.56	0.36[a]

[a] Statistics conducted on log-transformed data.
[b] Three of 4 gilts had no measurable CBG-like binding activity and were assigned values of zero for statistical evaluations.

31 of pseudopregnancy [29]. Because changes occurred in both cyclic and pregnant gilts, the conceptus is unlikely to be the source of these corticosteroids. Although extraadrenal sites for 11β-hydroxylase enzymatic activity and aldosterone synthesis have been demonstrated [30], the primary site of cortisol and aldosterone synthesis is the adrenal gland [31]; hence, the maternal adrenal cortex is presumably the ultimate source of these intraluminal corticosteroids. Since injections of hydrocortisone acetate for 10 days increased blood and intrauterine cortisol concomitantly in pseudopregnant pigs [29], such a proposed mechanism seems plausible, although additional mechanisms such as tissue metabolism by 11β-hydroxysteroid dehydrogenase [32, 33] may regulate entry of steroids into the uterine lumen. Because blood and tissue samples were obtained at slaughter with its numerous stressors and elevated ACTH, which would abnormally elevate glucocorticoids and aldosterone [34, 35], serum cortisol and aldosterone were not measured to determine their association with intraluminal corticosteroids. Of note in this respect is the fact that intrauterine progesterone was not correlated with serum progesterone, as was also previously reported [36].

These data are the first to demonstrate the presence of CBG-like activity within the porcine uterine lumen and endometrium. A discrepancy exists between immunologically reactive and biologically active CBG, for which there are two possible explanations. First, the antibody to porcine CBG might cross-react with other members of the SERPIN superfamily of proteins that are present within the porcine uterus [19–21]. This possibility cannot be totally excluded, but the antibody to porcine CBG was highly specific for most proteins and concentrations tested. Uteroferrin-associated protein with previously demonstrated purity [37] exhibited a low but measurable cross-reactivity. At Day 110 of pseudopregnancy, intraluminal uteroferrin-associated protein concentrations can achieve μg/μl levels [21], but concentrations at the time intervals studied are unknown. However, the endometrial message of uteroferrin-associated protein is much lower at Day 13 than at Days 70–100 of pregnancy [22], and its concentrations are unlikely to exceed those of immunoreactive uteroferrin itself, which are approximately 600 ng/ml on Days 12 and 16 of pregnancy [38]. The SERPIN protein plasmin, which was not tested

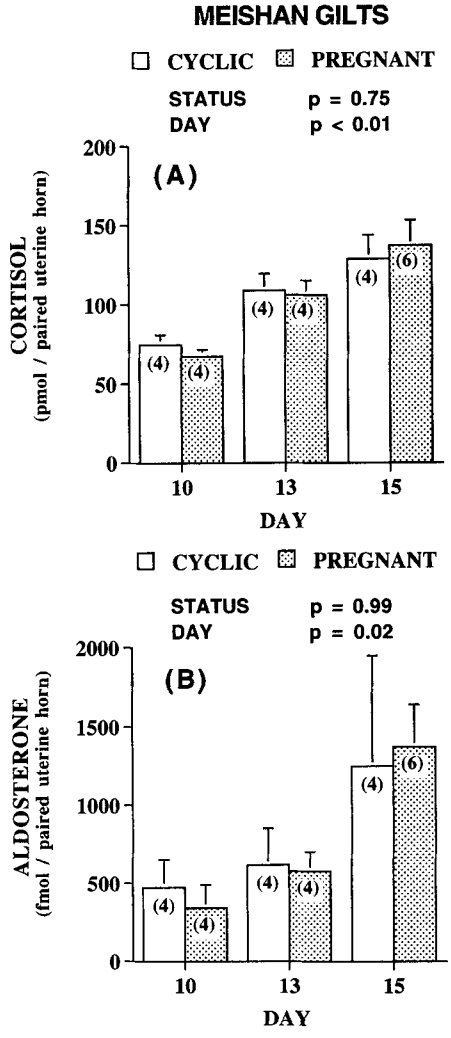

FIG. 7. A) Total cortisol content in uterine flushings from Chinese Meishan gilts during the estrous cycle and pregnancy. There was no status × day interaction ($p = 0.81$). Total aldosterone content in uterine flushings from Chinese Meishan gilts during the estrous cycle and pregnancy. There was no status × day interaction ($p = 0.63$). All statistics were conducted on $\log_{10}$ transformed data.

for cross-reactivity, decreases dramatically at about Day 14 [20], unlike ir-CBG in the current study.

A second possibility is that intraluminal CBG may be cleaved by proteases such that its biological activity was lost [39], but its immunological activity retained. Indeed, the polyclonal antibody was developed against two proteins (59 and 54 kDa) of identical amino terminal sequence [18]. The protein of higher molecular weight (~50%) retained its ability to bind cortisol, but that of lower molecular weight did not (unpublished results).

The source of uterine luminal and endometrial CBG-like binding activity is unknown. Blood contamination of the cytosolic preparations did not seem to occur, as evidenced by the absence of hemoglobin in our cytosolic preparations. It is probable that cytosolic preparations also contained interstitial fluid and could therefore contain CBG of blood origin. It is of interest to note, however, that decreases in endometrial CBG-like activity between Days 10 and 15 did not reflect the constant serum CBG concentrations. Increases in uterine flushing CBG-like binding activity concomitant with decreases in endometrial CBG-like activity suggest a secretion of CBG by the endometrium. However, only future measurements of CBG mRNA will be able to accurately determine whether CBG is synthesized within porcine endometrial cells. Moreover, although CBG binding capacity in uterine flushes is quite low even at Day 15 relative to cortisol levels, the increases (25- to 46-fold) of CBG-like activity between Days 10 and 13 are much greater than increases in uterine protein (4.4- to 6.6-fold). This suggests that increased intraluminal CBG-like activity is specific and does not merely represent a generalized increase in luminal proteins that might be associated with increased uterine capillary permeability [40]. Corticosteroid binding globulin has been previously measured in rodent [41, 42] and human [15] uterine tissues.

Whether endometrial CBG-like activity measured in the current study is intra- or intercellular, its presence suggests a specific role for this CBG-like activity in endometrial function. It is known that CBG and CBG receptors are present in many glucocorticoid and progesterone target tissues. Although their exact function is unknown, CBG receptors might direct progesterone—with which CBG also binds [16]—or corticosteroids to specific target tissues [17]. Thus, CBG-like activity within the porcine endometrium could mediate actions of either cortisol or progesterone. However, the measured K_d for progesterone in the current studies indicates a lower affinity for that steroid than previously shown for humans [16] and suggests that high concentrations of progesterone would be needed before effective competition with cortisol for CBG would occur.

Binding of tritiated cortisol in endometrial cytosol preparations could have represented glucocorticoid receptors instead of CBG. That this is not the case is evidenced by the fact that 1) dexamethasone, which is known to effectively compete for both corticosteroid type I and type II receptors [43, 44], but not CBG [45] (unpublished results), was unable to displace tritiated cortisol; and 2) aldosterone, which binds primarily type I receptors [44], displaced only 10% of tritiated cortisol binding in the endometrial cytosol. It is noteworthy that cytosolic binding of tritiated cortisol was displaced by aldosterone to a much lesser extent than that in serum. Hence, although serum, uterine flushing, and endometrial binding had similar K_ds, this differential displacement by aldosterone suggests somewhat different molecular characteristics.

Our data clearly demonstrate that the majority of cortisol within the porcine uterine lumen is present in the free state (Fig. 5), as was previously demonstrated by Behrens and coworkers [29]. Thus, if free cortisol represents its biologically active form [17], then intraluminal cortisol should be able to subserve a biological function. The exact nature of a function for intrauterine cortisol and aldosterone remains to be investigated, but two possibilities in the pregnant uterus are suggested: 1) cortisol modulation of embryonic erythropoiesis (as has been demonstrated in other species [46–48]), and 2) aldosterone regulation of sodium, potassium, and associated water transport across allantoic epithelial cells (as it is known to do in epithelial cells of the distal kidney tubules [49, 50]). Absence of information concerning their exact roles notwithstanding, the intrauterine presence and increase of these corticosteroids during early pregnancy suggest their involvement during early porcine conceptus development.

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